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Housing and Husbandry Conditions Affect Stereotypic Behaviour in Laboratory Gerbils

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Summary
An artificial burrow was developed which fits into standard laboratory cages and significantly reduces stereotypic digging in gerbils. Also, the causes of bar-chewing were assessed experimentally. Neither the lack of gnawing material, nor the spatial proximity of cage-lid bars and food in the hopper, nor the routine husbandry procedure of transferring juvenile gerbils to a fresh cage, but premature separation of juvenile gerbils from their parents before the birth of younger siblings significantly increased bar-chewing.

Keywords: gerbil, stereotypies, housing, enrichment, refinement

Background Information

Refinement of rodent husbandry conditions, a relevant issue?

- Animal welfare laws require that the environment of captive animals should meet their physiological and behavioural needs. Housing conditions must facilitate the performance of natural behaviour patterns and allow for adequate social contacts. Unfortunately little is done about behavioural and often the social needs of laboratory rodents, usually only the physiological needs are covered.
- Refinement of experimental procedures relies on animals free of chronic stress and abnormal behaviour. If housing conditions already represent a stressful situation, the validity of experimental results gained from such animals is questionable (Würbel, 2001; Garner, 2005).
- Standardisation of experimental procedures requires a reduction of intra- and interindividual variation. Notwithstanding, huge individual variation of stereotypic behaviour patterns (for example: bar-gnawing, jumping, somersaulting in laboratory mice) can be observed in animals under barren housing conditions, reflecting their different strategies to cope with the situation (Mason and Rushen, 2007). In this case the validity of experiments conducted on such animals is also affected.
- Current debate suggests that animals living in a more complex environment, as for example an enriched laboratory cage, show more natural behaviour patterns, exhibit less intra-individual variation and can cope better with novel situations, resulting in less stress reactions and robust results in standard behavioural paradigms, without masking phenotypic differences between strains (Wolfer et al., 2004).

Artificial housing conditions and behavioral abnormalities

It has long been known that inadequate housing and breeding conditions promote the development of abnormal behaviour such as stereotypies and replacement activities in farm, zoo and laboratory animals. The mechanism leading to behavioural abnormalities is hypothesised as follows: under natural conditions in the wild, animals interact with their biotic and abiotic environment, whereby internal and external stimuli serve to regulate the frequency of certain behaviour patterns. This enables organisms to adapt flexibly to differing environmental conditions. In domesticated animals, many behavioural mechanisms are still regulated in the same manner and by the same stimuli as in their wild ancestors. Under artificial housing conditions, the animals may not be able to adapt to the barrenness of their environment or cope with the lack of appropriate sets of regulating stimuli. As a result, they often show stereotypies, defined as the repeated performance of the same behaviour without apparent goal or function. In farm and zoo animals, stereotypies have successfully been treated by supplying the animals with an appropriate set of artificial environmental stimuli, also called environmental enrichment. In the laboratory, environmental enrichment has long been neglected and should be implemented more frequently.

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Investigations in laboratory gerbils

Mongolian gerbils (*Meriones unguiculatus*), which are often used for parasitological and neurological research, represent a useful model for stereotypies. Gerbils growing up under standard laboratory housing conditions typically develop two distinctive behavioural abnormalities: stereotypic digging and chewing on the bars of their cage. We analysed the causes of this behaviours by adding specific enrichment factors to the standard housing conditions or by modifying the handling of the gerbils during breeding. Afterwards we observed these animals weekly from their birth to adulthood to quantify the frequency and duration of bouts of stereotypic behaviour. In addition, we measured cortisol levels in fresh faeces collected once a week (ELISA) as an indicator of chronic stress, but found no increased cortisol levels depending on the housing conditions.

1. Simulation of burrows

In the wild, gerbils live in vast subterranean burrow systems which provide shelter against the climate and predators. Standard laboratory cages lack a burrow-like structure into which the animals can retreat. In such a situation, the gerbils are highly motivated to attempt to create a burrow by digging. Their efforts remain unsuccessful, which then leads to stereotypical repetition of digging motions (Wiedenmayer, 1997). Based on Wiedenmayer's results, we developed a prototype artificial burrow system integrated into a standard laboratory cage and tested its efficiency in reducing stereotypic digging. In gerbils grown up with such a burrow system stereotypic digging was significantly reduced.

2. Separation of the juveniles from their family

Initially we hypothesised that bar-chewing in gerbils was either a replacement activity caused by the lack of appropriate chewable nesting material or a reinforced bar-manipulation caused by the close proximity of their food pellets to the bars of the cage-lid in the food hopper. Our first experiments showed, however, that neither of these factors had an influence on the development of bar-chewing (Waiblinger and König, 1999; Fig. 2). Upon closer observation, we found that bar-chewing significantly increased after juvenile gerbils were routinely separated from their families and housed in a separate cage with fresh bedding material at the age of 35 days ("Separation" in Fig. 2; Waiblinger and König, 2004). In a second experiment, we therefore tested the influence of both the process of separation from their family and the transfer to a fresh cage on the development of stereotypic bar-chewing in a two-way factorial design. Juvenile gerbils significantly increased bar-chewing only if separated from their family before the next litter ("younger siblings" in Fig. 3) was born, but not after a transfer to a fresh cage together with their family or after a separation after the birth of younger siblings (Tab. 1). Bar-chewing might therefore reflect the juvenile animals' motivation to return to their families, as long as there are no younger siblings present. By separating family and juveniles only after the birth of a younger litter in the family, the development of stereotypic bar-chewing can thus be reduced ("Separation" in Fig. 2 and 3). The time interval between litters averages 35 days in gerbils, therefore we suggest not separating juveniles from their parents before the age of 5 weeks.

Simple refinement is effective

Our experiments have shown that rather simple refinement in animal housing conditions and flexible timing of separation of juveniles can successfully reduce or even prevent specific stereotypic

Tab.1: Results of a repeated measures ANOVA showing the effects of transfer (to a clean cage) and/or separation (from parents and siblings), weight at the age of 35 days, and presence of younger siblings (within subjects factors) on the development of bar-chewing before (days 33 and 34) and after transfer and/or separation (days 36 and 37); age was used as within-subjects factor.

Factor	F	df	P
age (days 36/37 compared to days 33/34)	**34.661**	**1**	**0.0001**
Transfer to a clean cage	*0.758*	*1*	*0.396*
Separation from parents and siblings	***7.180***	***1***	***0.016***
presence of pups, i.e. younger siblings	*0.018*	*1*	*0.895*
weight at 35 days	*1.959*	*18*	*0.086*
Age x transfer	0.017	1	0.898
Age x separation	**12.213**	**1**	**0.003**
Age x presence of pups	**4.594**	**1**	**0.047**
Age x weight	1.911	18	0.094
Age x transfer x separation	0.133		0.720
Age x transfer x presence of pups	0.003		0.957
Age x transfer x weight	0.887		0.493
Age x separation x presence of pups	2.160		0.160
Age x separation x weight	**3.355**		**0.023**
Age x presence of pups x weight	**3.169**		**0.041**

Between subjects factors are indicated in italics, significant effects are indicated in bold type.

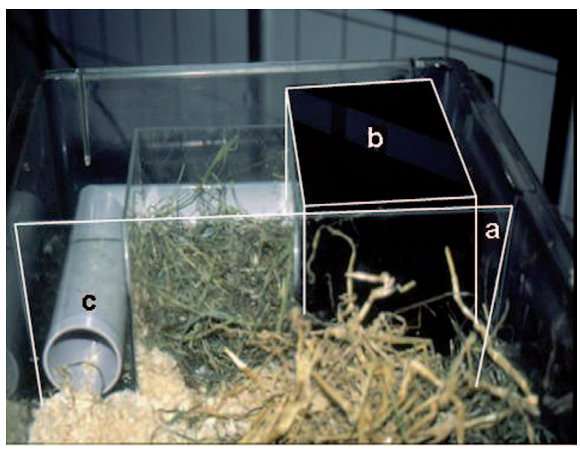

Fig. 1: Prototype of an artificial burrow that consists of three modules (a: separation wall, b: dark nestbox with removable lid and c: access tunnel) and can be easily integrated into a standard Makrolon cage type IV.

behaviour. Delayed separation of the juveniles will not affect other aspects of animal management since juvenile gerbils do not usually reproduce as long as they remain with their parents.

Stereotyping animals are poor models for biomedical research

Stereotypies are not only aberrant behaviours or bad habits, but indicate more in-depth abnormalities in the central nervous system of animals performing stereotypies. Garner (2005) showed that stereotyping animals exhibit cognitive deficits such as behavioural disinhibition similar to schizophrenic human patients if tested in appropriate behavioural paradigms such as the spatial extinction task (i.e. the animal has to learn that a previously learnt path is not rewarded any more). If such animals are used in behavioural tests that require any form of learning and extinction of learning, activity, response latencies or behavioural variability, then stereotyping animals will perform much poorer than non-stereotyping animals. Also, this suggests that there are underlying neuronal bases in stereotyping and behaviourally disinhibited animals, i.e. changes in neuronal pathways and brain metabolism, which make these animals poor models in biomedical research.

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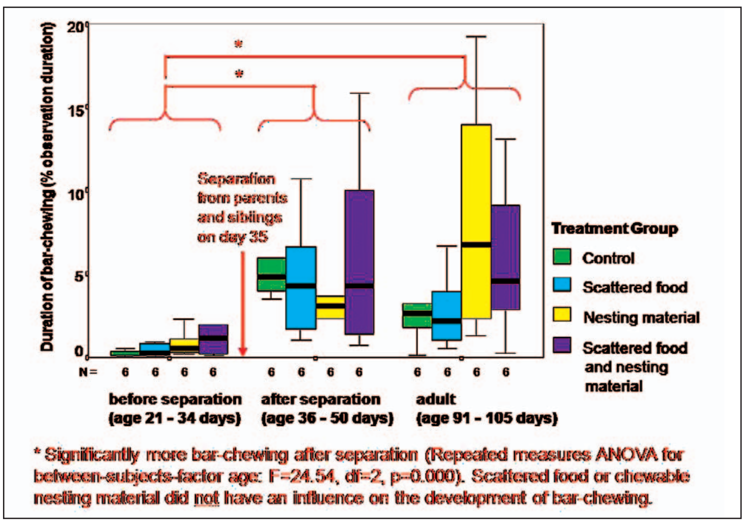

Fig. 2: First experiment.
Influence of age, chewable nesting material and scattered food in bedding on the development of bar-chewing in juvenile laboratory gerbils. The latter factors do not have an influence on the development of bar-chewing (measured over a 42 min observation period/animal/2 days once a week from day 21 to day 105 after birth). However, bar-chewing increased significantly after the gerbils had been separated from their parents and siblings and been placed in isosexual groups of two in clean cages – a normal husbandry procedure.

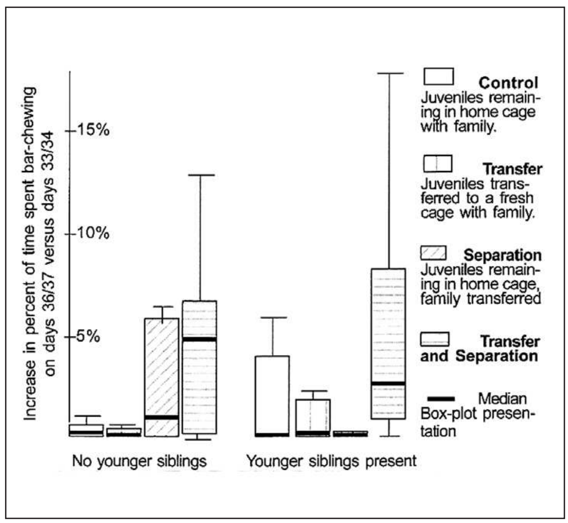

Fig. 3: Second experiment.
Influence of separation from parents and siblings, transfer to a clean cage and the presence of younger siblings on the development of stereotypic bar-chewing measured over a 21 min observation period/animal/day on day 33/34 and 36/37. Positive values mean an increase; negative values a decrease in bar-chewing.